IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Rajagopalan et al.
Serial No.:	09/898,809
Filed:	July 3, 2001
Group Art Unit:	1624
Confirmation No:	5120
Examiner:	McKenzie
Title:	**DYE-SULFENATES FOR DUAL PHOTOTHERAPY**
Our Ref. No.:	MRD-63

Cincinnati, Ohio 45202 October 25, 2005

Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JOHN K. BUOLAMWINI, Ph.D.
PURSUANT TO 37 C.F.R. §1.132

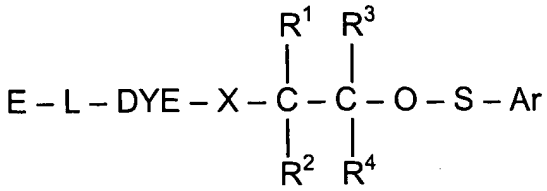
I, John K. Buolamwini, declare as follows:

I am a Medicinal Chemist and hold the rank of Full Professor in the Department of Pharmaceutical Sciences at the University of Tennessee, Memphis College of Pharmacy. My Curriculum Vitae is attached. I have read the specification of U.S. Patent Application Serial No. 09/898,809 as it was filed with the U.S. Patent and Trademark Office, the claims currently pending, and the July 25, 2005 Office Action.

I understand that the Examiner holds the opinion that the specification does not disclose sufficient information to put the public in possession of the

invention, which is referred to as the "written description" requirement in the Office Action. I understand that a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventors had possession of the claimed invention. I understand that possession can be shown with words, structures, figures, diagrams, and structural chemical formulas. I understand that actual reduction to practice is not required. I understand that the Examiner holds the opinion that the specification does not enable those skilled in the art to make and use the invention, as it is claimed, without undue experimentation, which is referred to as the "enablement" requirement in the Office Action. I understand its purpose is to ensure that the invention is communicated to the interested public in a meaningful way, and that the specification must be sufficient to inform those skilled in the relevant art how to both make and use the claimed invention. I understand that factors to be considered include the breadth of the claims, the nature of the invention, the state of the prior art, the level of one of ordinary skill, the level of predictability in the art, the amount of direction provided by the inventors, the existence of working examples although I understand that working examples are not required, and the quantity of experimentation needed to make or use the invention based on the content of the disclosure. I understand that the Examiner holds the opinion that the claims are not sufficiently definite because one skilled in the art would not know identities of the claimed receptor binding compounds.

The instant application claims a method of performing a photosensitizing procedure in which the sulfenate, as part of the formula



is administered to a target tissue, and then the target tissue is exposed to light at a wavelength, power and fluence rate to cause necrosis or apoptosis of the target tissue.

Based on the documents that I reviewed, I was asked if and how I could determine each of the following:

- the relationship between the structure of the sulfenate and its function,
- selection of the targeting group and attachment to the sulfenate,
- what compounds or portions of compounds to select as a binding molecule for each of the following receptors: somatostatin, heat sensitive bacterioendotoxin, neurotensin, bombesin, cholecystekinin, steroid, and carbohydrate
- how I would carry out the method that is claimed.

I understand that the Examiner finds the application does not describe and/or enable the above issues, and for this reason the Examiner rejects the application.

I respectfully disagree with the Examiner. It is my opinion that, based on the patent application as filed, I am able to address each of the above issues as I subsequently explain. It is my opinion that doing so requires a level of experimentation that is reasonable for one skilled in this art; it is not "undue".

I do not agree that if a molecule has been reported as a ligand to a receptor there is no correlation between structure and function. It is precisely because of the structure that the molecule binds to the receptor; the binding establishes a structure/function relationship. Molecules fitting the "receptor binding" claim terms are those known to bind the receptor at the time of invention. This is not indefinite, as the literature at the time reveals all such

reported molecules. More specifically, at the time of the invention there were definite numbers of known or reported ligands or binding molecules for all the receptors as the claims recite. I now describe some of these, and attach the appropriate citations.

The estrogen receptor is an example of a steroid receptor to which "steroid receptor binding molecules" would bind. The following compounds are known to bind to the estrogen receptor: estratriol, 17β-aminoestrogen (AE) derivatives such as prolame and butolame; drugs such as tamoxifen, ICI-164384, raloxifene, genistein; 17β-estradiol; glucocorticoids, progesterone, estrogens, retinoids, fatty acid derivatives, phytoestrogens, etc. Further, there are commercially available kits that identify compounds specific for binding to the estrogen receptor (e.g., Estrogen Receptor-alpha Competitor Assay Kit, Red; Estrogen Receptor-beta Competitor Assay Kit, Red (Invitrogen Corp., Carlsbad CA).

The glucose receptor is an example of a carbohydrate receptor to which "carbohydrate receptor binding molecules" would bind. The glucose conjugate N-palmitoyl glucosamine [NPG] is known to bind the glucose receptor (Dufes et al.. Pharm. Res. 17:1250-1258, 2000). The glycoprotein hormone receptor is another example of a carbohydrate receptor to which "carbohydrate receptor binding molecules" would bind. Follicle stimulating hormone (FSH) is known to bind the glycoprotein hormone receptor (Tilly et al., Endocrinology 131: 799, 1992). Other compounds known to bind the carbohydrate receptor, and hence examples of "carbohydrate receptor binding molecules", are polysialic

acid, bacterial adhesins (specialized surface proteins that mediate binding of many pathogenic bacteria, such as enterohemorrhagic *E. coli* (EHEC) and *Shigella dysenteria*, to host cells, which allows these bacteria to colonize host cell surfaces), soluble carbohydrate receptor analogs, artificial glycopolymers and other multivalent glycoconjugates such as an acrylamide copolymer carrying α-L-fucopyranoside and 3-sulfo-β-D-galactopyranoside in clusters, isomeric carbohydrates, synthetic derivatives, neoglycoproteins, and neoglycolipids. It has also been reported that carbohydrate binding proteins can be screened with phage display libraries.

"Somatostatin receptor binding molecules" include somatostatin and somatostatin analogs, octreotide, glycosylated somatostatin-14 (somatostatin-dextran^{70}), seglitide, peptides P587 and P829 as described in Vallabhajosula et al., J. Nuclear Med., 37:1016, 1996.

"Cholecystekinin receptor binding molecules" include the endogenous peptides cholecystekinin (CCK)-4, CCK-8, CCK-33, and gastrin; antagonists devazepide and lorglumide; agonists BC264 [Tyr(SO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_3] and desulfated CCK-8; Kinevac (synthetic cholecystekinin, sincalide); and CCK analogues modified at the sulfated tyrosyl at position 27.

"Neurotensin receptor binding molecules" include neurotensin, neuromedin N, JMV449 (*H*-Lysψ(CH_2NH)-Lys-Pro-Tyr-Ile-Leu), the non-peptide antagonist SR142948A (2-([5-(2,6-dimethoxyphenyl)-1-(4-(*N*-[3-dimethylaminopropyl]-*N*-methylcarbamoyl)-2-isopropylphenyl)-1*H*-pyrazole-3-

carbonyl)amino)adamantine-2-carboxylic acid hydrochloride), and levocobastine. Further, there are neurotensin receptor binding kits to evaluate potential neurotensin receptor binding compounds (e.g., DELFIA Neurotensin Receptor Binding Kit, PerkinElmer (Boston MA)).

"Bombesin receptor binding molecules" include the endogenous ligands gastrin-releasing peptide (GRP), neuromedin B (NMB), and GRP-18-27, and antagonists including JMV-1458 (glycine-extended bombesin (paraphydroxy-phenyl-propionyl-Gln-Trp-Ala-Val-Gly-His-Leu-Met-Gly-OH)), PD165929, 1-naphthoyl-[DAla^{24},DPro^{26},ψ26-27]GRP-20-27, kuwanon H, and kuwanon G. Further, there are bombesin receptor binding kits to evaluate potential bombesin receptor binding compounds (e.g., DELFIA Bombesin Receptor Binding Kit, PerkinElmer (Boston MA)).

I therefore disagree with the Examiner that the structure of the targeting group was indefinite at the time of the invention. As a medicinal chemist who makes molecules that bind primarily to receptors or enzymes, I cannot immediately profane a molecule that binds to a receptor unless I have seen that molecule described as a ligand for the receptor, or I myself have made such a molecule. In the former case I can propose a potential ligand that will be a derivative or analog of the already known molecule. That does not mean that the molecule does not exist, however, and it does not mean that I cannot, by a single literature search, uncover it. It is reasonable that a chemist or medicinal chemist will perform a literature search to find a molecule that will bind a receptor. I agree that there are many carbohydrate receptors, but the Examiner's statement

that there are possibly thousands or millions of receptor types whose identity is "unknown or unknowable" is inconsistent with what we know about receptor types. This appears to be an exaggeration. Currently, I do not know more than about ten high affinity selective receptor types for any particular ligand. I do not agree with the Examiner that somatostatin receptor binding molecule, etc. are not art-recognized structural terms. When one hears these as a medicinal chemist, one can envision that there might already exit such molecules or they could be discovered. For example, E could be an antibody or part of a monoclonal antibody–FAB fragment; there are methods for linking antibodies to other compounds, etc.

With respect to the relationship between the structure of the sulfenate and its function, the sulfenate is a photoreactive compound that will undergo homolytic cleavage upon irradiation with electromagnetic radiation to produce free radical species that can destroy tissues or cells. The choice of E will depend on the receptor to which E should bind, which in turn is dictated by the disease to be targeted.

Knowing this, I would perform a literature search to find molecules (ligands) reported to bind to the chosen receptor. Once I have chosen the desired molecule based on its high affinity for the receptor, I would perform a chemical reaction to link the molecule to the dye with a linker, also linking the dye to the sulfenate. For example, FIG. 3 is useful and teaches me to be mindful of stoichiometry and controlled addition of components due to the presence of the

two activated succinimidyl esters, otherwise, two molecules of the sulfenate will couple to the cyanine dye.

Upon performing my literature search, I would identify molecules with high affinity binding, preferably having low nanomolar K_d values, and molecules that have a structure such that they can be conjugated to the dye, which has activatable carboxylic acid groups, such as, compound 3 in FIG. 3. For example, I would be looking for compounds having a primary amino group that is not stearically hindered and use this group to link to the dye. If such a compound is not available, I would engineer a handle with a spacer on the ligand molecule at the appropriate substitution position. This might take some time to do. It may be that I have to perform screening assays to discover such a compound and use it, which is quite feasible.

To carry out the claimed method, I would decide for which disease to use phototreatment. I would then determine which receptors are highly or selectively expressed in that tissue or, if cancer, the cancer cells. I would then select a targeting molecule or ligand to that receptor (i.e. E) then link E to the dye, which is linked to the sulfenate, as shown in FIG. 3. Companies sell such linking agents.

After linking E to DYE, which is linked to the sulfenate, I would purify it, then assay binding with the receptor preferably expressed on the tissues or cells I intend to destroy in the photo procedure. I would use a known binding molecule (ligand) to the receptor as a competitive ligand to see how well it is displaced by the E-DYE-sulfenate. From this experiment, I would determine the

binding affinity in the form of a K_d value. The competitive ligand could be the molecule E that was coupled to the dye. The attachment of E to the dye and sulfenate might affect the binding affinity of E to the receptor, and this experiment would show whether or not E still binds strongly enough to the receptor to make the product useful for the photo procedure. Thus, it is my opinion that one skilled in the art would immediately recognize competitive assay as one type of binding assay.

Once satisfied that the product binds sufficiently to the receptor, I would make a pharmaceutical formulation of the compound, apply it to the tissues at the doses indicated, wait for the appropriate time to allow the compound to bind to the tissues or cells, and then irradiate the tissues, for example, with a fiber optic tube using a laser at the specified wavelengths. Other considerations may be toxicity of the product to the host or patient, which would be determined by the stability of the sulfenate as well as the targeting group or ligand E and may not be a concern.

Locating the claimed formula at the receptor so that the sulfenate can be photoactivated is required; the inventors are not claiming any particular binding property. Thus, I respectfully disagree with the Examiner's statement that "Since the binding affinities of molecules for receptors are dependent upon the conditions of the assay such information is crucial for determining which molecules are embraced by Applicants' claims". The binding assays will be performed under physiological conditions of buffer pH and temperature etc. In my opinion, binding affinities and assay conditions are not "crucial" for determining E

for at least two reasons: (1) any binding that sufficiently locates the sulfenate at the claimed receptors will suffice; and (2) one skilled in the art knows or can reasonably determine without undue experimentation which compounds will locate the sulfenate at the claimed receptors, as I have previously described.

It is my opinion that the state of the art is well developed to make the claimed compounds and perform the claimed methods. The descriptions can be understood by anyone skilled in the art of medicinal chemistry. Ordinary chemists and medicinal chemists would be able to put a composite molecule together, and pharmaceutical scientists would be able to formulate the compound for administration. The experimentation involved is with the literature search to identify the binding molecules (E), attaching E to the sulfenate and dye, determining binding affinity, and optimizing spacer length to enhance binding affinity. It is my opinion that such experimentation is familiar to a medicinal chemist and is not undue experimentation.

I respectfully disagree with the Examiner that there is no correlation between the structure of the claimed formula and its function. The specification discloses that the sulfenate group is key to the photoactivating process. Free radicals are produced upon exposure to light of the proper wavelength. E is the group that binds to one of the claimed receptors and hence locates the formula at the desired site.

Based on the teachings of the specification, I understand that E would serve to locate the active portion of the molecule to the "target" site to be treated. This target is a cell or tissue containing a receptor for one of the

compounds listed above. Thus, a compound that binds to one of these receptors would locate the sulfenate to the desired site. As described, the results of my literature search would allow me to envision the molecules that would bind to each of these receptors. The selection, addition, and evaluation of such a targeting compound is not, in my opinion, "undue" experimentation because the identity, availability, affinity, testing, etc. of such receptor binding compounds are established in the art. In my opinion, any experimentation to formulate or enhance such targeting would certainly not be "undue", but instead would be encompassed by routine organic synthesis and/or receptor binding assays as I have described.

For at least the reasons I have set forth, I respectfully assert that one skilled in the art would be able to make the claimed invention without undue experimentation, and that the inventors were in possession of their invention at the time they filed the instant application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the subject application or any patent issued thereon.

October 25, 2005 10/25/05
Date

John K. Buolamwini, Ph.D.